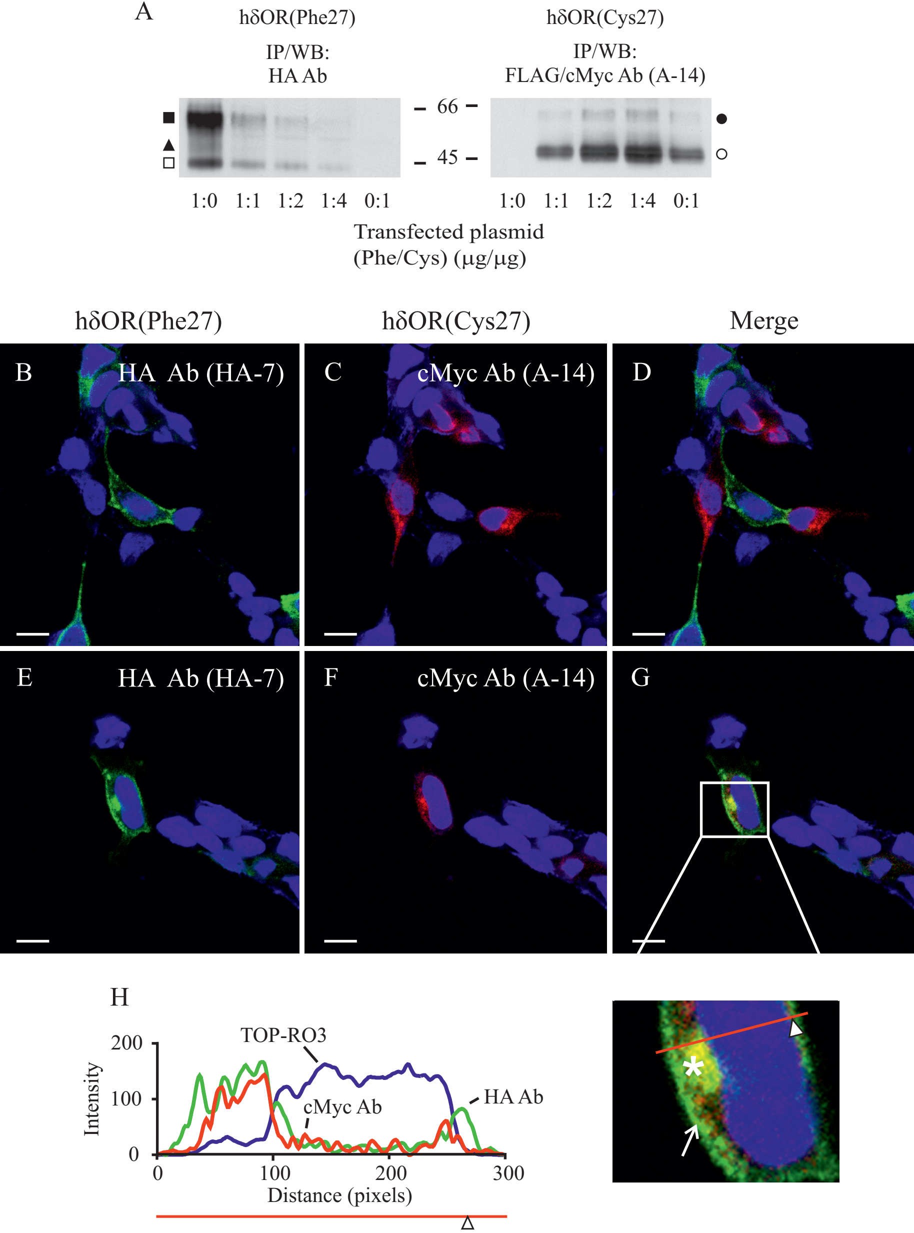
SUPPLEMENTAL FIGURES

Supplemental Figure 1. Subcellular localization of hδOR^{Cys27} and hδOR^{Phe27} in transiently co-transfected Flp-in-293 cells. *A*, Flp-in-293 cells were co-transfected with HA-hδOR^{Phe27} and Myc-hδOR^{Cys27}-FLAG using various cDNA ratios, as indicated. After 24 h, solubilized receptors from cellular lysates were subjected to immunoprecipitation, SDS-PAGE and Western blotting using the indicated antibodies. The precursor and mature receptor forms are depicted with open and closed symbols, respectively: (□, ■) hδOR^{Phe27} with two *N*-glycans, (▲) hδOR^{Phe27} with one *N*-glycan, (○, ●) hδOR^{Cys27} with two *N*-glycans. *B-G*, Flp-in-293 cells were transfected with HA-hδOR^{Phe27} and Myc-hδOR^{Cys27}-FLAG (1:2 ratio) following protocols that favored expression of only one (*B-D*) or both (*E-G*) variants in individual cells. After 24 h, the cells were fixed and stained with HA (green) and cMyc (red) antibodies followed by Alexa Fluor 488/568 secondary antibodies, respectively. Nuclei were stained with TOP-RO3 iodide (blue). Cells were analyzed by confocal microscopy. The arrow indicates plasma membrane receptors and the asterisk intracellular receptor accumulation. The red line with an arrowhead indicates the line selection used for the RGB profile plot analysis shown in panel *H.* Bars, 10 μm. In cells expressing only hδOR^{Phe27} or hδOR^{Cys27} (*B-D*), the former localized mainly at the cell surface, while the latter was predominantly intracellular. In co-transfected cells (*E-G*), the variants co-localized in a compact perinuclear compartment.

Supplemental Figure 2. Co-localization of hδOR^{Phe27} with ER, ERGIC and Golgi markers in SH-SY5Y neuroblastoma cells co-transfected with hδOR^{Cys27} and hδOR^{Phe27}. SH-SY5Y neuroblastoma cells were transfected with HA-hδOR^{Phe27} and Myc-hδOR^{Cys27}-FLAG (1:2 ratio) for 24 h following a method that resulted in efficient co-expression of both variants in individual cells. Co-immunostaining of hδOR^{Phe27} and various marker proteins was performed after fixation and permeabilization, using mouse monoclonal (*A-F*) or rabbit polyclonal (*G-L*) HA antibodies and the relevant marker antibodies, as indicated, followed by Alexa488/568-conjugated secondary antibodies. Nuclei were stained with TOP-RO3 iodide (blue). Cells were analyzed by confocal microscopy. The intracellularly accumulated receptors co-localized weakly with ER resident proteins calreticulin and Sec61β (*A-F*) and more extensively with ERGIC and Golgi markers ERGIC-53 and GM130, respectively (*G-L*).

Supplemental Fig 1.



Supplemental Fig 2.